REMARKS

Upon entry of the amendments herein, claims 1-12, 14 and 15 are pending in the application. Claims 1-10 and 14 have been amended; claim 13 has been canceled; and new claim 15 has been added. The amendments have been made to more clearly recite the subject matter regarded as the invention and/or to correct informalities; to reintroduce amendments originally made in the parent application; and in response to various indefiniteness rejections leveled by the Examiner. New claim 15 replaces canceled claim 13 and is directed to the same subject matter. Also, the specification has been amended by insertion of a cross-reference to priority applications. No new matter has been introduced by any of the amendments herein.

The Examiner has asserted that the present title is not descriptive and required a new one. A new title along the lines of that suggested by the Examiner has been added by amendment herein.

Claims 5-7, 10 and 14 have been objected to as being improperly multiply dependent. These claims have been amended to overcome the objection.

Claim 1 has been rejected as indefinite for containing the term "BSSL" without further definition. The claim has been amended to recite what the term represents.

Claim 2 has been rejected as indefinite for containing the phrase "substantially similar." The cited language has been removed from the claim, and the rejection is moot.

Claims 1 and 4 have been rejected as indefinite for containing the phrase "biologically active variant." The phrase has been removed from claims 1 and 4, as well as 8 and 14, and replaced with language that recites more clearly and particularly what is meant by the phrase; the rejection is now moot.

Claim 4 has also been rejected as being indefinite for failing to clearly describe what a variant of human BSSL is limited to in terms of deletions and for containing the phrase "being indicated in." Both of these grounds for rejection are inappropriate and should be withdrawn.

As disclosed in the specification on page 2, lines 16 and 17, "[T]he C-terminal region of the protein contains 16 repeats of 11 amino acid residues each...." The Feature sections for SEQ ID NO: 1 in the Sequence Listing identify or "indicate" (the language presently used in the claim) the locations of each of the 16 repeat units. Claim 4 in its present form is unambiguously directed to a variant of full-length BSSL that lacks one or more of these repeat units. It must also be noted, though, that these "repeat units" are not all identical in sequence. The only difference between the Examiner's understanding of the invention claimed in claim 4 and that intended is that the variants do not result from "at least one deletion of the repeat unit" (emphasis added), but deletion of "at least one of" the units identified in the final 16 Feature sections of SEQ ID NO: 1. This "difference" in interpretation is certainly not the fault of the claim language.

Furthermore, and in any case, the Examiner's suggested phrase "the repeat unit consisting of SEQ ID NO: 1" is completely inappropriate in the context of the present invention. SEQ ID NO: 1 contains the entire sequence coding for BSSL and, as such, includes the sequences encoding the various 11-amino-acid repeat units. Again, the locations of these sequences are pinpointed in the Features section for SEQ ID NO: 1 in the Sequence Listing. Thus, again, there is no ambiguity in the meaning of the claim, and the cited language is particularly appropriate in view of the fact that the repeat units are not all identical.

Certainly, the Examiner's suggestion does nothing to clarify the meaning of the claim; in fact, the Examiner's suggestion would signify that any one of the sixteen 11-amino-acid repeats is SEQ ID NO: 1. However, each of said repeats is simply a discretely defined segment of the larger molecule defined in SEQ ID NO: 1. Contrary to the Examiner's assertion, the "metes and bounds" of the claim in its present form are clear.

Claims 1-4 have been rejected as being nonenabled by the disclosure of the instant specification. The Examiner asserts: "However, only the DNA sequence encoding human BSSL has been disclosed. Those DNA encoding a biologically active variant of human BSSL have not been disclosed." The Examiner further maintains that "the disclosure fails to describe the common attributes or characteristics that identify members of the genus...."

In the first place, the claims have been amended to define the scope of the variants in terms of percent homology with the

wild-type enzyme and to more particularly recite the biological activity attributed to the variants. Furthermore, contrary to the Examiner's assertion, the specification does disclose the identity and expression of a variant BSSL.

Not only has efficient expression of the wild-type (full-length) BSSL been demonstrated in *Pichia pastoris* using the native signal sequence (Examples 1 and 2), but efficient expression at high levels of the BSSL-C variant (with deletions from residues 536 to 568 and 591-711), this time using the heterologous *Saccharomyces cerevisiae* invertase SUC2 signal sequence, has also been demonstrated (Example 4). This is adequate support for the contention that Applicant's inventive system can be used for the efficient expression of both full-length and variant BSSL's.

Still further, as the Examiner himself has been wont to assert, BSSL variants of the type to be expressed according to the instant invention were previously disclosed. Applicant's invention is not the variant molecules themselves but a novel, unexpected way of efficiently expressing them using systems employing constructs containing, inter alia, the variant molecules. It is inconsistent for the Examiner to invoke prior disclosure for the purpose of an obviousness rejection but ignore the same disclosure in making an enablement rejection. Applicant cannot be required to reteach what is already known.

Thus, adequate disclosure of "what characteristics could distinguish compounds in the genus" and more than "a single

disclosed member of the genus" have been provided. Withdrawal of the rejection is respectfully requested.

Claims 1-4 have been rejected as being nonenabled for containing the phrase "functionally equivalent promoter." The deletion of this phrase renders moot the rejection.

Claims 1-4 have been rejected under the judicially created doctrine of obviousness-type double patenting as being obvious over claim 1 of U.S. Patent No. 5,827,683 in view of disclosure found in European Application No. 0 438 200 of Martinez, et al. The Examiner essentially maintains that one of skill in the art would have been motivated to combine the '683 teaching of BSSL variants wherein repeat units are deleted with Martinez disclosure of an expression vector for achieving expression and secretion of proteins in *P. pastoris* and thus arrive at the instant invention. However, the Examiner has merely identified prior art purported to disclose each of the individual features of the instant invention and has asserted that it would have been obvious for one of skill in the art to combine these disparate teachings. In making this assessment, the Examiner has ignored the key features of the instant invention and the overall understanding in the state of the art at the time of the instant invention.

The instant invention is directed to enhancing recombinant expression of BSSL and active variants thereof by expressing the gene encoding such a protein in *P. pastoris* cells under the control of the methanol oxidase promoter. The key technical features are the methanol oxidase promoter and the use of *P.*

pastoris as host. Whether or not BSSL variants containing deletions were previously known is immaterial to the assessment of the patentable distinctness of the instant invention; Applicant is not claiming the variants. Instead, what is claimed is an unexpectedly successful method of expressing the variants employing constructs comprising, inter alia, sequences encoding the variants. A nucleic acid molecule encoding a given polypeptide cannot reasonably be thought of as patentably indistinct from a nucleic acid molecule which is an expression vector capable of expressing the encoded polypeptide.

As disclosed in the instant specification (page 4, lines 5-9), successful expression of a heterologous protein in active, soluble and secreted form depends on a variety of unpredictable factors, e.g., the correct choice of signal peptide and so on. Although a number of signal peptides and yeast expression systems were known at the time of filing of the instant application, it would not have been predictable which ones would allow correct and efficient high level expression of the active polypeptide.

The Examiner's assessment that the teaching of Martinez would automatically have motivated one of skill in the art to use the Martinez expression system for expression of any heterologous protein is borne of failure to consider the unpredictability known to exist in the field at the time of the instant invention. By the Examiner's reasoning, in light of some published accounts of success with systems using *S. cerevisiae* as host cell for the expression of heterologous proteins, one might have been "motivated" to use this organism as the host.

In fact, however, when Applicant indeed tried using *S. cerevisiae* as host for the expression of BSSL, he found it to be ineffective (see page 18 of the instant specification). It was found that the expression level in *S. cerevisiae* was too low to be quantified and that the native signal sequence was not cleaved, with the inevitable result of intracellular sequestration of expressed protein. The observation that BSSL could not be efficiently expressed in *S. cerevisiae* with a native signal sequence notwithstanding, Applicant found, surprisingly, that efficient expression could be achieved in *P. pastoris* with a native signal sequence or in *P. pastoris* with an *S. cerevisiae* signal sequence.

If, in fact, it were obvious to use *P. pastoris* as the host for expression of human BSSL, then surely using *S. cerevisiae* would also have been obvious at the time of the instant invention. However, it did not prove to be the case that efficient high level expression of BSSL in *S. cerevisiae* could be achieved, and, therefore, success in effecting such expression in *P. pastoris* is no more *prima facie* obvious, i.e., expected, than the use of any other host and expression vector system for the purpose of producing large quantities of functional human BSSL.

In an earlier enablement rejection in the present Office Action, the Examiner invoked "quantity of experimentation necessary" as a basis for rejecting the scope of certain claims. Yet, the Examiner chose not the bear in mind this very same consideration in merely lumping together various teachings in the prior art and asserting that it would have been obvious to do so

at the time. Such inconsistent analysis is particularly inappropriate in view of the known unpredictability, and even discouraging results, known to those working in the field.

Applicant's success in devising a successful system for expression of human BSSL, despite the lack of success with a "tried and true" expression system and the general unpredictability with respect to expression systems, is indeed unexpected. Withdrawal of the rejection is respectfully requested.

Claims 1, 2 and 4 have been rejected under 35 USC §103(a) as being obvious over U.S. Patent No. 5,200,183 to Tang, et al. in view of U.S. Patent No. 4,808,537 to Stroman, et al.; and claim 3 has been rejected under the same statute as being obvious over the Tang patent in view of the previously cited European Application of Martinez, et al. The same considerations that apply to the analysis of the obviousness-type double patenting rejection also apply here. Again, the mere throwing together of different disclosures, and the assertion that it would have been obvious at the time to do so, do not stand up to the unpredictability in the art and the equally significant discouraging results known to those working in the field. For the same reason that the obviousness-type double patenting rejection should be withdrawn, so too should the §103(a) rejections.

Applicant also wishes to remind the Examiner of the contradiction that results from citing the Tang reference at all as prior art to the instant application. The Tang reference was

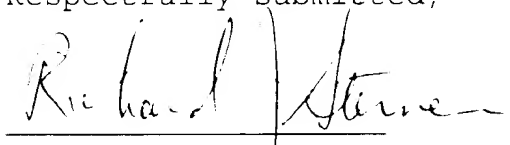
also cited during prosecution of the application from which Patent No. 5,827,683 issued, but the Examiner ultimately acknowledged the patentable distinctness of said application over Tang. However, the Examiner now not only reinvokes Tang in a 103 a rejection, but cites the '683 patent in an obviousness-type double patenting rejection of the instant application.

In view of the amendments herein to the claims and the arguments set forth above, the subject matter regarded as the invention is clearly recited and said subject matter is fully enabled. Furthermore, the claims are directed to subject matter which is patentably distinct over the cited prior art. Reconsideration and allowance of pending claims 1-12, 14 and 15 are respectfully requested. Should any other matters require attention prior to allowance of the application, it is requested that the Examiner contact the undersigned.

The Assistant Commissioner is hereby authorized to charge any fees which may be due for any reason to Deposit Account No. 23-1703.

Dated: June 13, 2001

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE

1. (amended) A DNA molecule comprising:

(a) a region coding for a polypeptide which [is] has human bile salt-stimulated lipase (BSSL) [or a biologically active variant thereof] activity and has an amino acid sequence which is at least 90% homologous with the sequence according to SEQ ID NO: 3 or SEQ ID NO: 4;

(b) a coding region, joined to the 5'-end of said polypeptide coding region, [a region] coding for a signal peptide capable of directing secretion of said polypeptide from *Pichia pastoris* cells transformed with said DNA molecule; and

(c), operably-linked to [said] the coding regions defined in (a) and (b), the methanol oxidase promoter of *Pichia pastoris* [or a functionally equivalent promoter].

2. (amended) A DNA molecule according to claim 1 wherein the [said] signal peptide has an amino acid sequence which is identical to[, or substantially similar to, the peptide with] the amino acid sequence shown as amino acids -20 to -1 of SEQ ID NO: 2 in the Sequence Listing.

3. (amended) A DNA molecule according to claim 1 wherein the [said] signal peptide comprises a *Saccharomyces cerevisiae* invertase signal peptide.

4. (amended) A DNA molecule according to any one of claims 1 to 3 encoding a [biologically active variant of] <u>polypeptide with</u> human BSSL <u>activity</u> in which at least one of the repeat units of 11 amino acids, said repeat[ed] units being indicated in SEQ ID NO: 1, is deleted.

5. (amended) A DNA molecule according to any one of claims 1 to [4] <u>3</u> coding for a polypeptide which has BSSL activity and <u>has</u> an amino acid sequence which is at least 95% homologous with the sequence according to SEQ ID NO: 3 or SEQ ID NO: 4.

6. (amended) A DNA molecule according to any one of claims 1 to [5] <u>3</u> coding for a polypeptide which has the amino acid sequence according to SEQ ID NO: 3 or SEQ ID NO: 4.

7. (amended) A vector comprising a DNA molecule according to any one of claims 1 to [6] <u>3</u>.

8. (amended) A replicable expression vector according to claim 7 which is capable of mediating expression [of human BSSL,

or a biologically active variant thereof,] in *Pichia pastoris* cells <u>of a polypeptide with human BSSL activity</u>.

9. (amended) A vector according to claim 8 which is the plasmid vector pARC 5771 [(NCIMB 40721)], pARC 5799 [(NCIMB 40723)] or pARC 5797 [(NCIMB 40722)].

10. (amended) Host cells of the genus *Pichia* transformed with a vector according to [any one of claims 7 to 9] <u>claim 7</u>.

14. (amended) A process for the production of a polypeptide which [is] <u>has</u> human BSSL <u>activity</u>, [or a biologically active variant thereof,] which comprises culturing host cells according to [any one of claims 10 to 13] <u>claim 10</u> under conditions whereby said polypeptide is secreted into the culture medium, and recovering said polypeptide from the culture medium.